E.Coli - CUPID

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WHY

E. Coli - CUPID ?

Cuprum Indicator

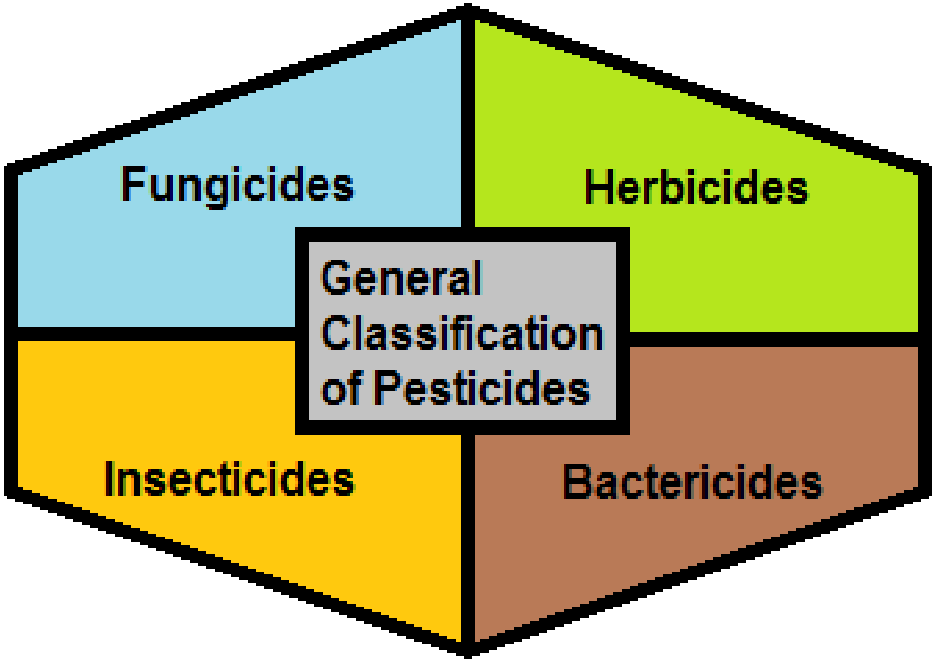
FOOD DEMANDS

VS

INTENSIVE FARMING

CHEMICALS PESTICIDES USAGE

Chemicals Pesticides :
A chemicals compound used for destroying insects or other organisms harmful to cultivated plants or to animals



- **Advantages**: Quick result and Easy to apply
- **Disadvantages**: High intensity usage is toxic for human and environment

Copper based pesticides is one of the most used pesticides due to broad spectrum function

Copper-based pesticide	Use pattern(s)
Copper (metallic)	Algaecide, antifouling paint
Copper (metallic in the form of chelates of copper citrate and copper gluconate)	Algaecide, bactericide, fungicide
Copper carbonate	Algaecide, herbicide, wood preservative
Copper ethanolamine complex	Algaecide, wood preservative
Copper ethylenediamine complex	Herbicide
Copper hydroxide	Antifouling paint, bactericide, fungicide, plant growth regulator, wood preservative
Copper naphthenate	Wood preservative
Copper oxychloride	Algaecide, bactericide, fungicide
Copper salts of fatty and rosin acids	Bactericide, fungicide
Copper sulfates	Algaecide, bactericide, desiccant,fungicide, herbicide
Copper triethanolamine complex	Algaecide
Copper oxides	Algaecide, antifouling paint, wood preservative

- **Copper Hydroxide**
  Agrocide 77WP,
  Champion77WP, Kocide54WG
- **Copper Oxide**
  Nordox 56WP & 86WP
- **Copper Oxine**
  Hylite 150EC
- **Copper Hexa Fluorocylicate**
  Cefka 97SP
- **Copper Oxichloride**
  Kasumin 5/75WP, Probox 50WP
- **Copper Oxisulphate**
  Sultricob 93 WP
- **Copper Sulphate**
  Betawood 97WG

COPPER EXCESSIVE SYMPTOMS in PLANTS

Chlorosis

Necrosis

Silene vulgaris **shows that soil contains high concentrations of cuprum**

COPPER TOXICITIES in HUMAN

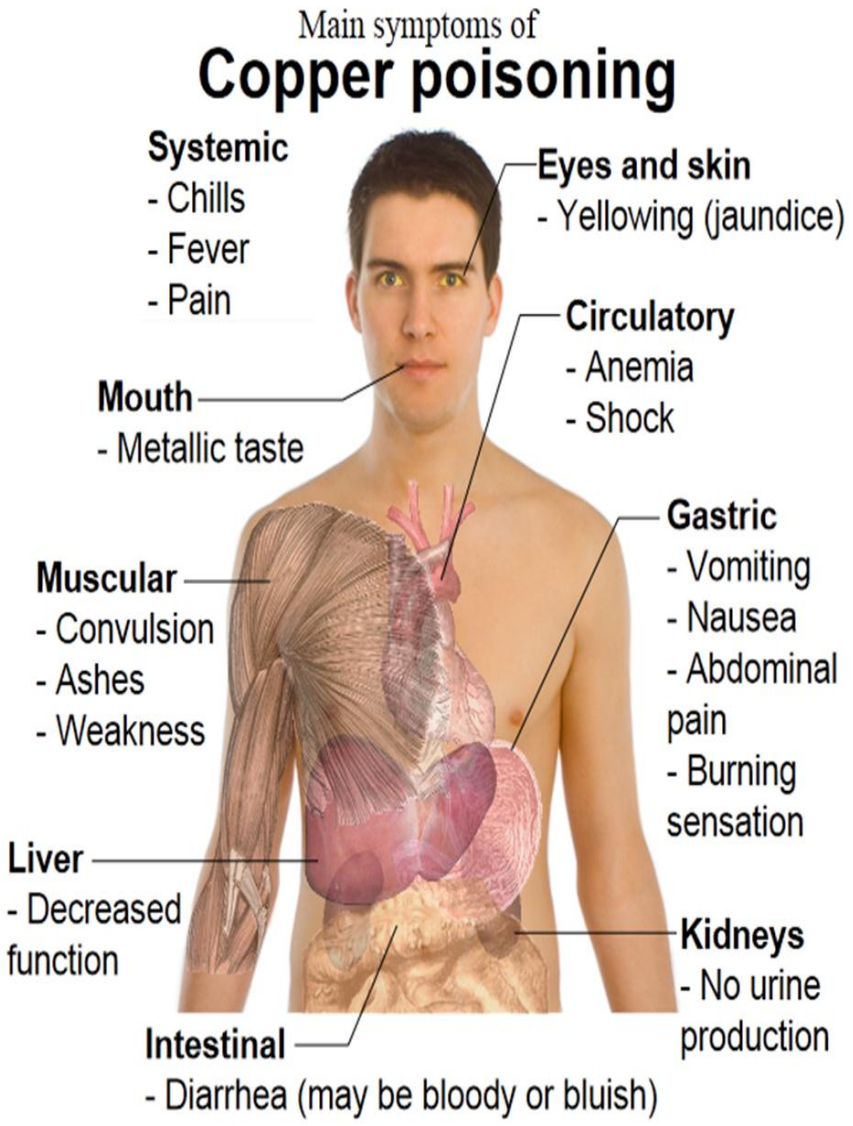


Table 4.1 Critical tissue concentrations for Cu toxicity in a variety of species

Species	Plant part	Critical toxicity ($mg.kg^{-1}$)	Method of cultivation	Reference
Twelve spp. of tropical grass	Shoots	18[1]	Soil pot experiment	(Plenderleith and Bell, 1990)
A. hypogaea	Shoots	230[2]	Soil pot experiment	(Borkert *et al.*, 1998)
Carthamus tinctorius (safflower)	Leaves	10[1]	Sand culture	(Pandey and Sharma, 1999)
G. max	Shoots	140[2]	Soil pot experiment	(Borkert *et al.*, 1998)
O. sativa	Whole plant	35[3]	Solution culture	(Lidon and Henriques, 1992)
O. sativa	Shoots	<20[2]	Soil pot experiment	(Borkert *et al.*, 1998)
T. aestivum	Shoots	75[4]	Solution culture	(Wheeler and Power, 1995)
	Roots	300		
Vigna mungo (blackgram)	Leaves	67[1]	Soil pot experiment	(Kalyanaraman and Sivagurunathan, 1993)
	Stems	50		
	Roots	41		

[1] Tissue concentration at 90% of maximum yield

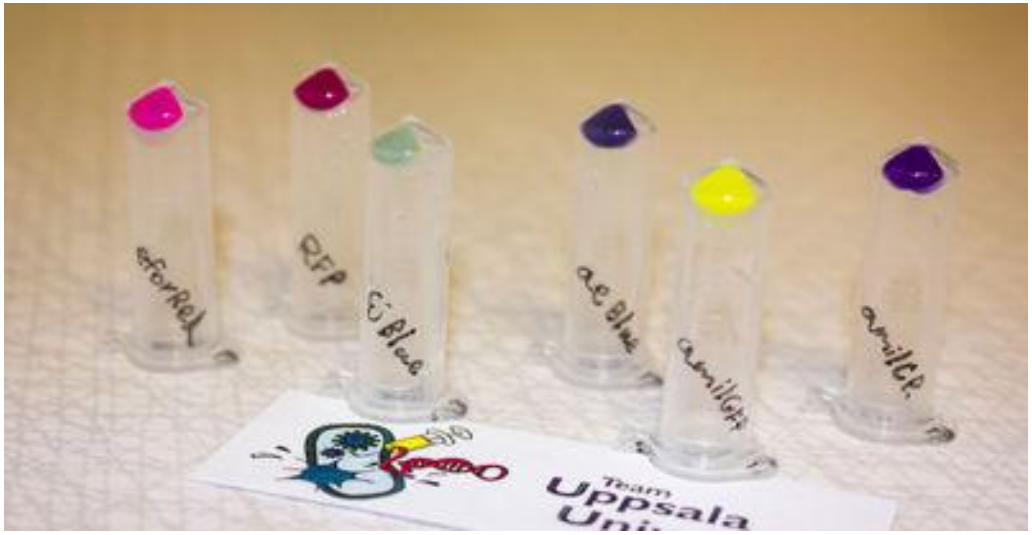
[2] Intersection of regression lines: "shoot growth independent of leaf metal concentration" and "growth inhibition at higher metal levels"

[3] Nutrient calibration curve of for total tissue concentration and root growth plotted on a log-log graph

[4] Estimated as a narrow range of plant concentrations where relative yield decreases rapidly

Bio Brick Design:

E. Coli - CUPID



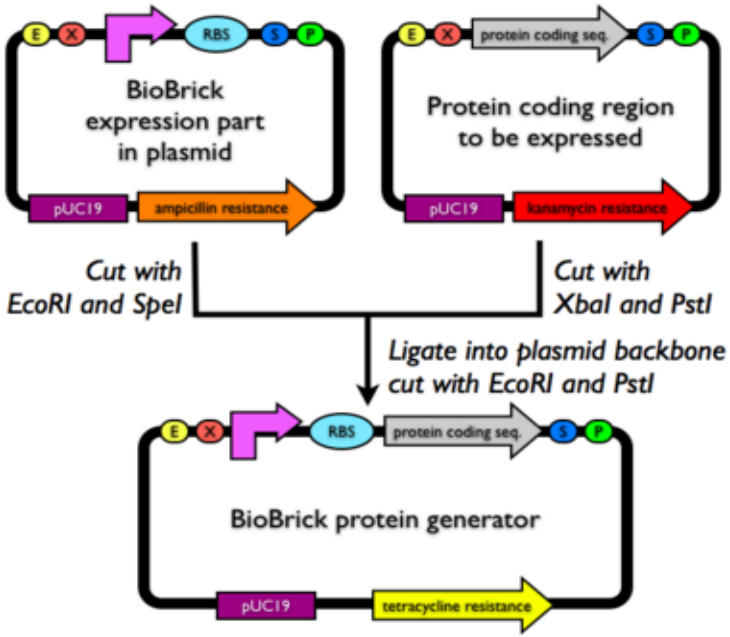
OBJECTIVES:

COPPER INDICATOR IN WATER

USING ENGINEERED *Escherichia coli*

PARTS USED:

Design *E. coli* CUPID using assembly method RCF 10



Forward terminators

More...

-?-		Name	Description	Direction	Efficiency Fwd.	Efficiency Rev.	Chassis	Length
1☆		BBa_B0010	T1 from E. coli rrnB	Forward				80
A	X	BBa_B0012	TE from coliphage T7	Forward	0.369[CC]	-0.368[CC]		41
A	X	BBa_B0013	TE from coliphage T7 (+/-)	Forward	0.6[CC]	-1.06[CC]		47
1☆	W	BBa_B0015	double terminator (B0010-B0012)	Forward	0.984[CC] 0.97[JK]	0.295[CC] 0.62[JK]	E.coli	129
1☆		BBa_B0017	double terminator (B0010-B0010)	Forward				168

PARTS USED:

Protein coding sequences/Selection markers

< Back to Protein coding sequences

Selection markers are protein coding sequences that confer a selective advantage or disadvantage to host chassis. For example, a common type of prokaryotic selection marker is one that confers resistance to a particular antibiotic. Thus, cells that carry the selection marker can grow in media despite the presence of antibiotic. Most plasmids contain antibiotic selection markers so that researchers can ensure that the plasmid is maintained during cell replication and division. (Cells that lose a copy of the plasmid will soon either die or fail to grow in media supplemented with antibiotic.) A second common type of selection marker, often termed a positive selection marker, are those that are toxic to the cell. Positive selection markers are frequently used during cloning to select against cells transformed with the cloning vector and ensure that only cells transformed with a plasmid containing the insert.

More...

-?-		Name	Protein	Description	Tag	Direction	UniProt	KEGG	Length
1☆		BBa_T9150	PyrF	orotidine 5	None	Forward	P08244	eco:b1281;	741
1☆	W	BBa_J31002	AadA-bkw	kanamycin resistance backwards (KanB) [cf. BBa_J23012 & BBa_J31003]			P0AG05	none	816
A		BBa_J31003	AadA2	kanamycin resistance forward (KanF) [cf. BBa_J23012 & BBa_J31002]			P0AG05	none	816
A		BBa_J31004	CAT-bkw	chloramphenicol acetyltransferase (backwards, CmB) [cf. BBa_J31005]			P62577	none	660
1☆	W	BBa_J31006	TetA(C)-bkw	tetracycline resistance protein TetA(C) (backwards) [cf. BBa_J31007]			P02981		1191
1☆	W	BBa_J31005	CAT	chloramphenicol acetyltransferase (forwards, CmF) [cf. BBa_J31004]			P62577	none	660
1☆	W	BBa_J31007	TetA(C)	tetracycline resistance protein TetA(C) (forward), [cf. BBa_J31006]			P02981		1191
1☆		BBa_K145151		ccdB coding region					306
A	W	BBa_K143031		Aad9 Spectinomycin Resistance Gene					771
1☆		BBa_K156011		aadA (streptomycin 3'-adenyltransferase)					789
A	W	BBa_K389005		Kanamycin resistance					819

PARTS USED:

Promoters/Catalog/Metal sensitive

This set includes promoters that are sensitive to various metals. The promoters are typically regulated by a receptor protein that binds to the metal ion or complex. There are promoters that are sensitive to iron, lead, and copper.

More...

-?-		Name	Description	Promoter Sequence	Positive Regulators	Negative Regulators
1☆		BBa_I721001	Lead Promoter	. . . gaaaaccttgtcaatgaagagcgatctatg		
		BBa_I760005	Cu-sensitive promoter	atgacaaaattgtcat		
A		BBa_I765007	Fe and UV promoters	. . . ctgaaagcgcataccgctatggaggggggtt		
1☆		BBa_J3902	PrFe (PI + PII rus operon)	. . . tagatatgcctgaaagcgcataccgctatg		

Anderson RBS family members

Identifier	Sequence[a]	Predicted Strength[b] Mean	CV
Master Sequence	TCTAGAGAAAGANNNGANNNACTAGATG		
BBa_J61101	TCTAGAGAAAGACAGGACCCACTAGATG		

Part:BBa_K592010

Designed by: Lei Sun Group: iGEM11_Uppsala-Sweden (2011-09-18)

amilGFP

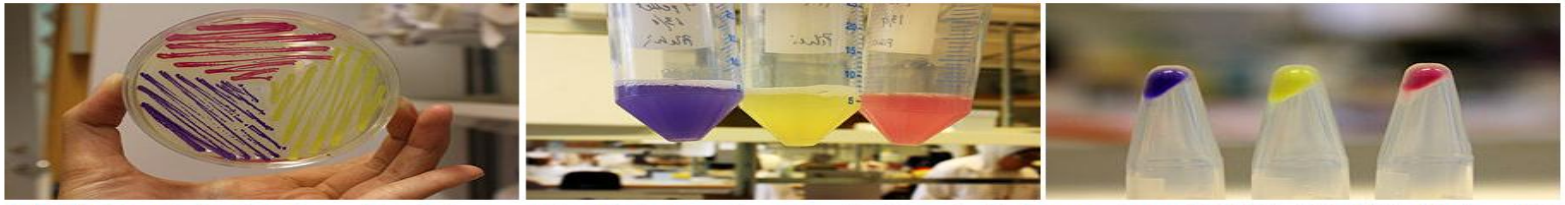
Not Released
Sample Pending
Experience: Works
8 Uses
Get This Part

amilGFP, yellow chromoprotein

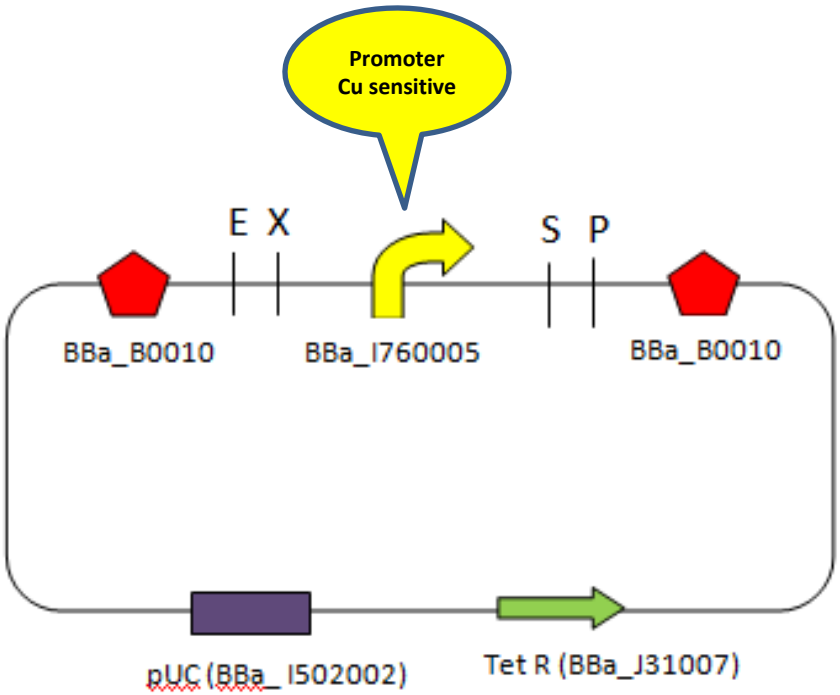
This chromoprotein from the coral *Acropora millepora*, amilGFP, naturally exhibits strong yellow color when expressed. The color is readily visible to naked eye both in LB-culture and on agar plates. Color development can be seen in less than 24 hours of incubation.

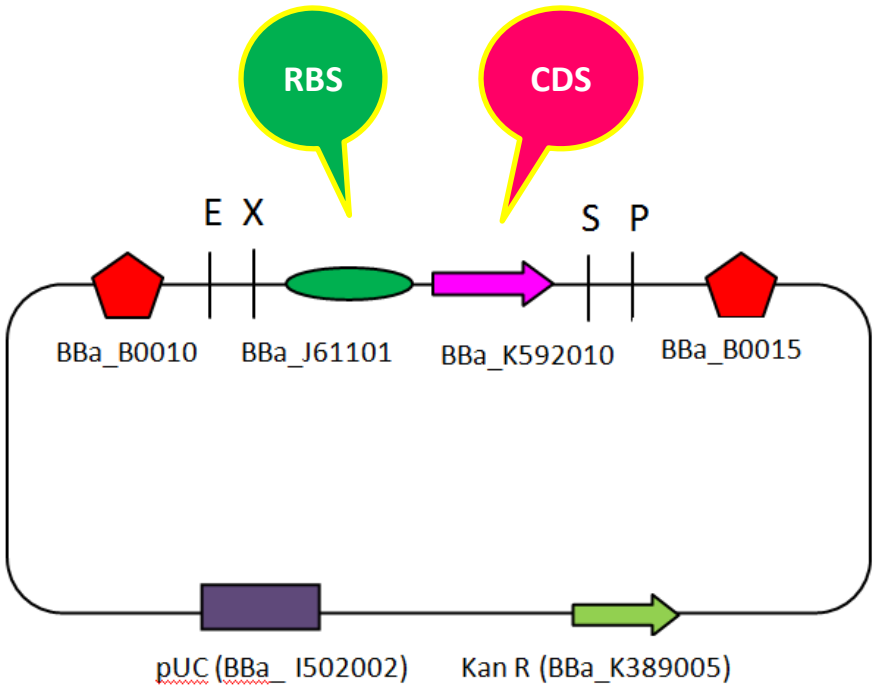
Usage and Biology

This part is useful as a reporter.

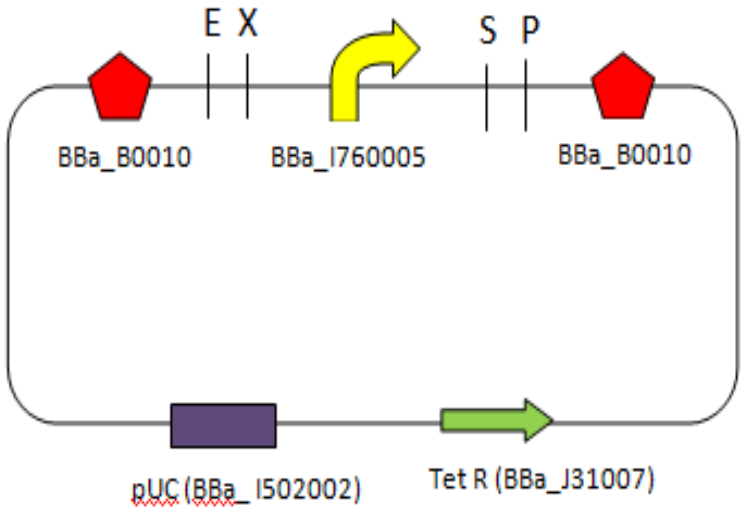


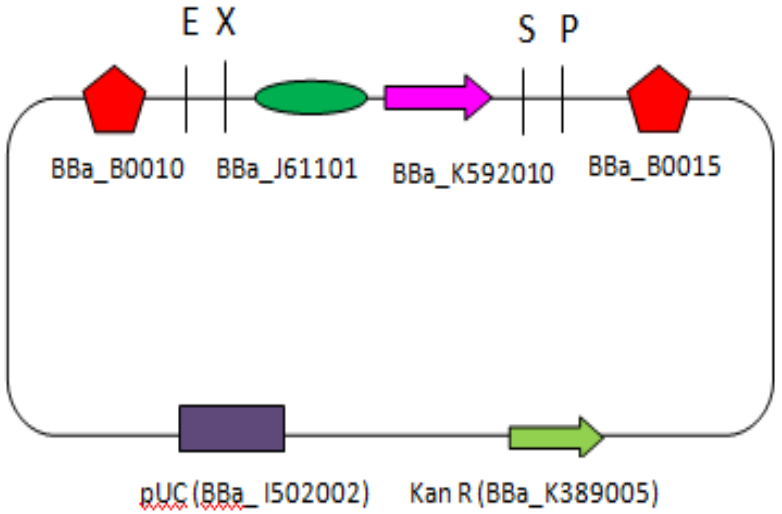
iGEM11_Uppsala-Sweden: Expression of chromoproteins. The images above show *E coli* constitutively expressing amilGFP BBa_K592010 (yellow), amilCP BBa_K592009 (blue), and RFP BBa_E1010 (red).


Promoter
Cu sensitive
E X
S P
BBa_B0010
BBa_I760005
BBa_B0010
pUC (BBa_ I502002)
Tet R (BBa_J31007)

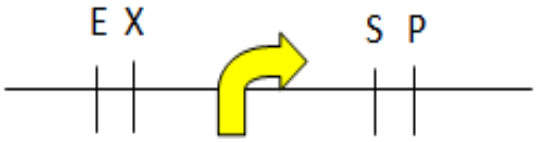

RBS
CDS
E X
S P
BBa_B0010
BBa_J61101
BBa_K592010
BBa_B0015
pUC (BBa_ I502002)
Kan R (BBa_K389005)

Desain Bio Brick CUPID

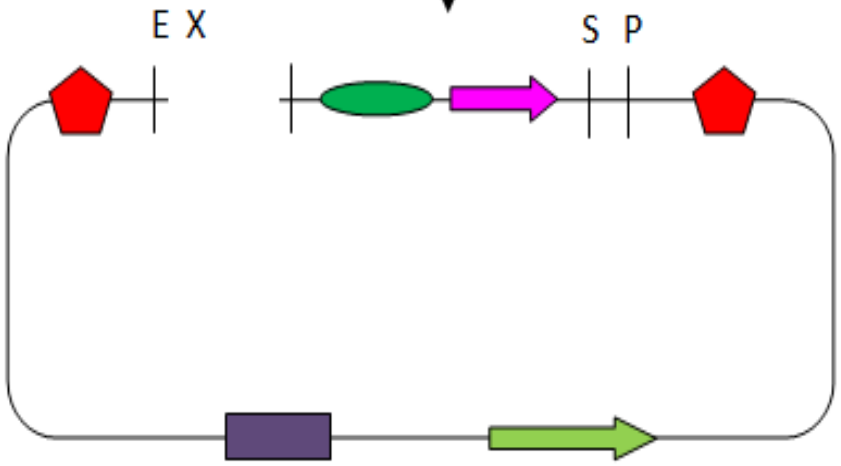




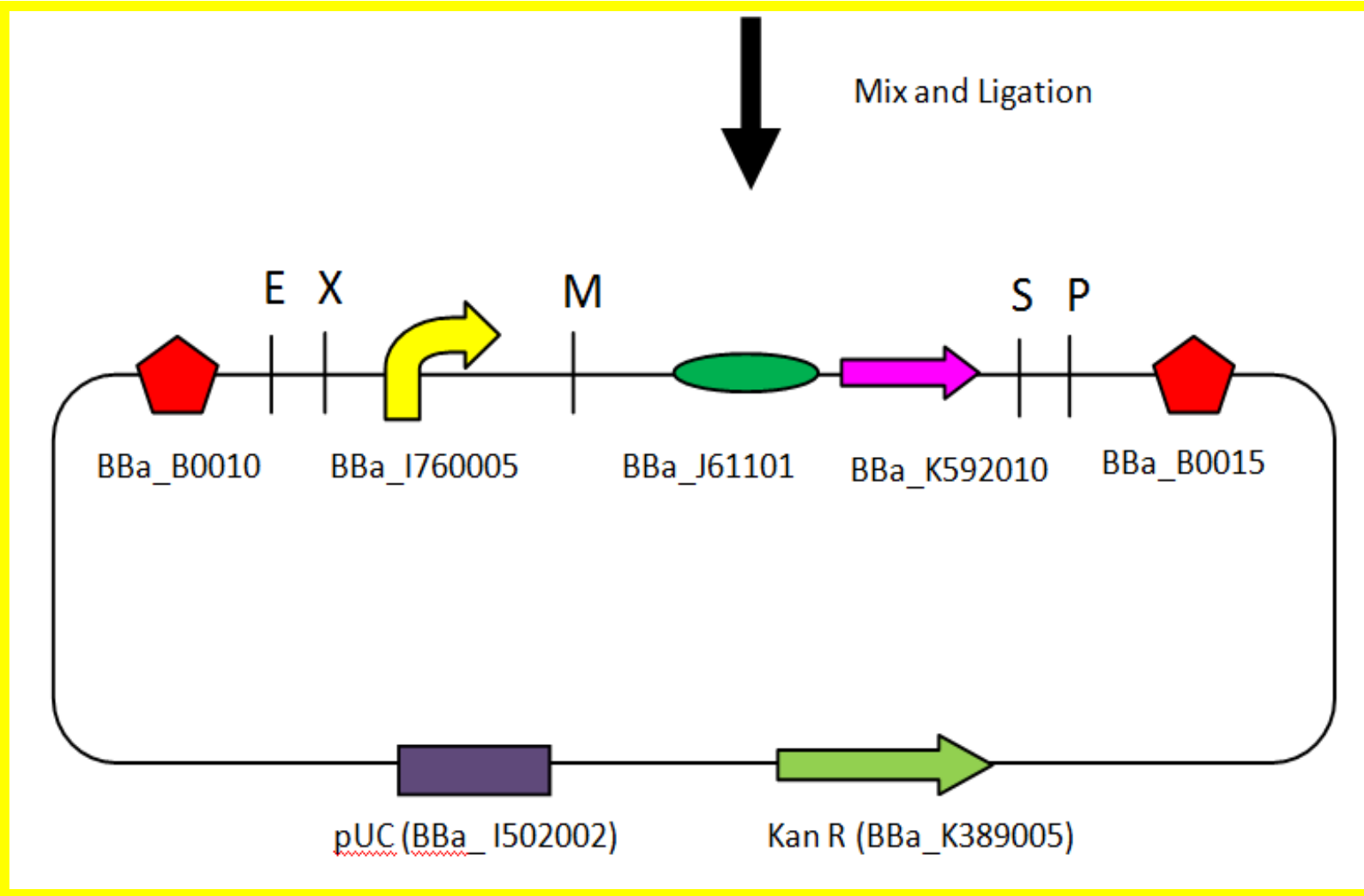
Cut with EcoRI and SpeI



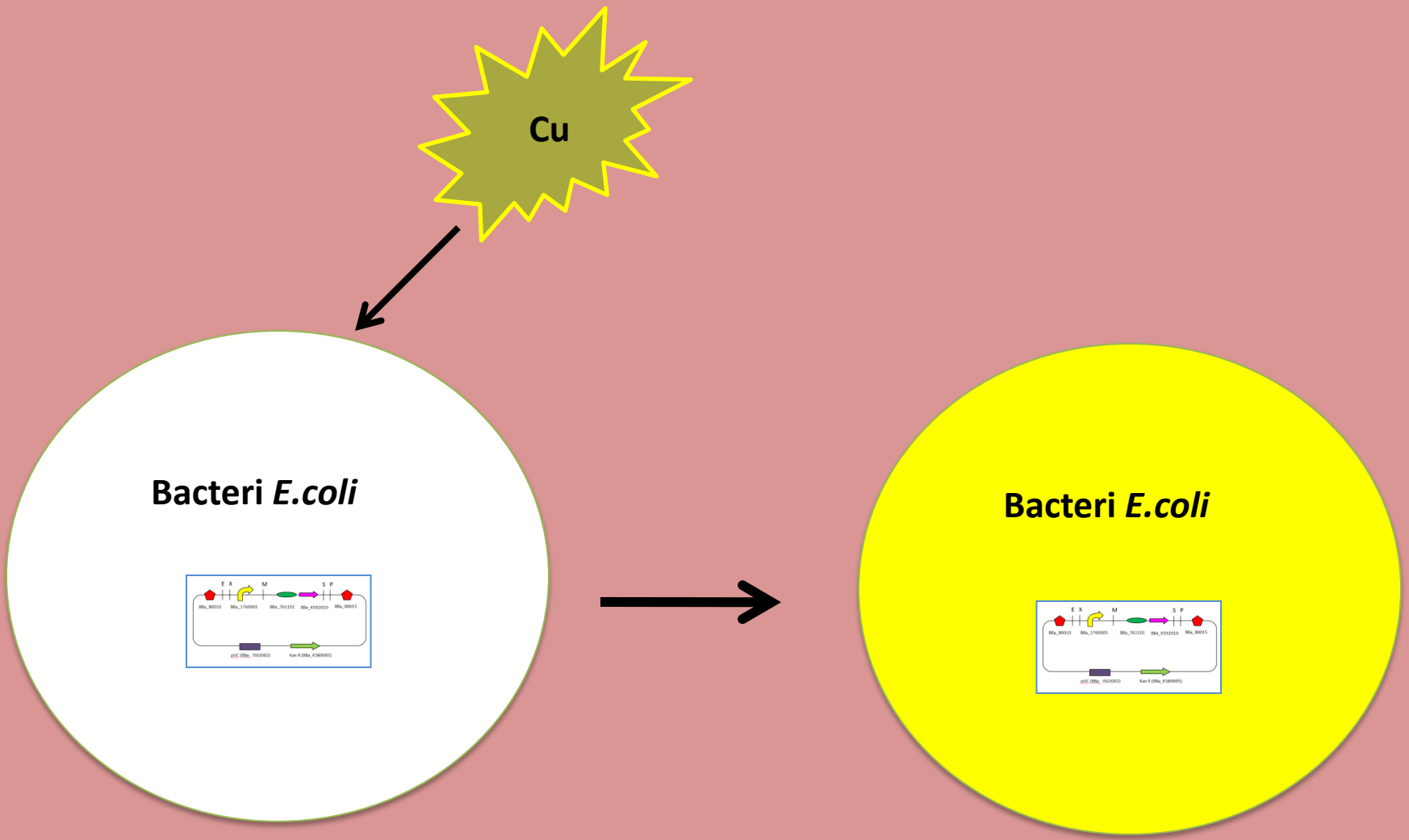
Cut with EcoRI and XbaI



Bio Brick Design:



Overview CUPID System



FUTURE DEVELOPMENT

FUTURE RESEARCH:

- Quantitative Measurement of copper content
- To enhance *E. coli* resistance to high excessive copper

FUTURE PRODUCT

EASY AND **CHEAP** KIT

Blotter paper color indicator

REFERENCES

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Franke. S., et al, 2003. Molecular Analysis of the Copper-Transporting Efflux System CusCFBA of *Escherichia coli*. Journal of Bacteriology: 3804- 3812

Rademacher. R., et al. 2012. Review: Copper-responsive gene regulation in bacteria. Microbiology 158:2451-2464

Reichman. S. M., 2002. The responses of plants to Metal Toxicity: A review focusing on Copper, manganese and Zinc. Australian Minerals & Energy Environment Foundation

World Health Organization. 2004. Copper in drinking water. WHO publication

Stern. B. R.,et al. 2007. Copper and Human Health: Biochemistry, Genetics, and Strategies for Modelling Dose response Relationships. Journal of Toxicology B.10:157-222

Http://parts.igem.org/main_page


THANK YOU

TAMBAHAN KALAU ADA PERTANYAAN YANG BERHUBUNGAN

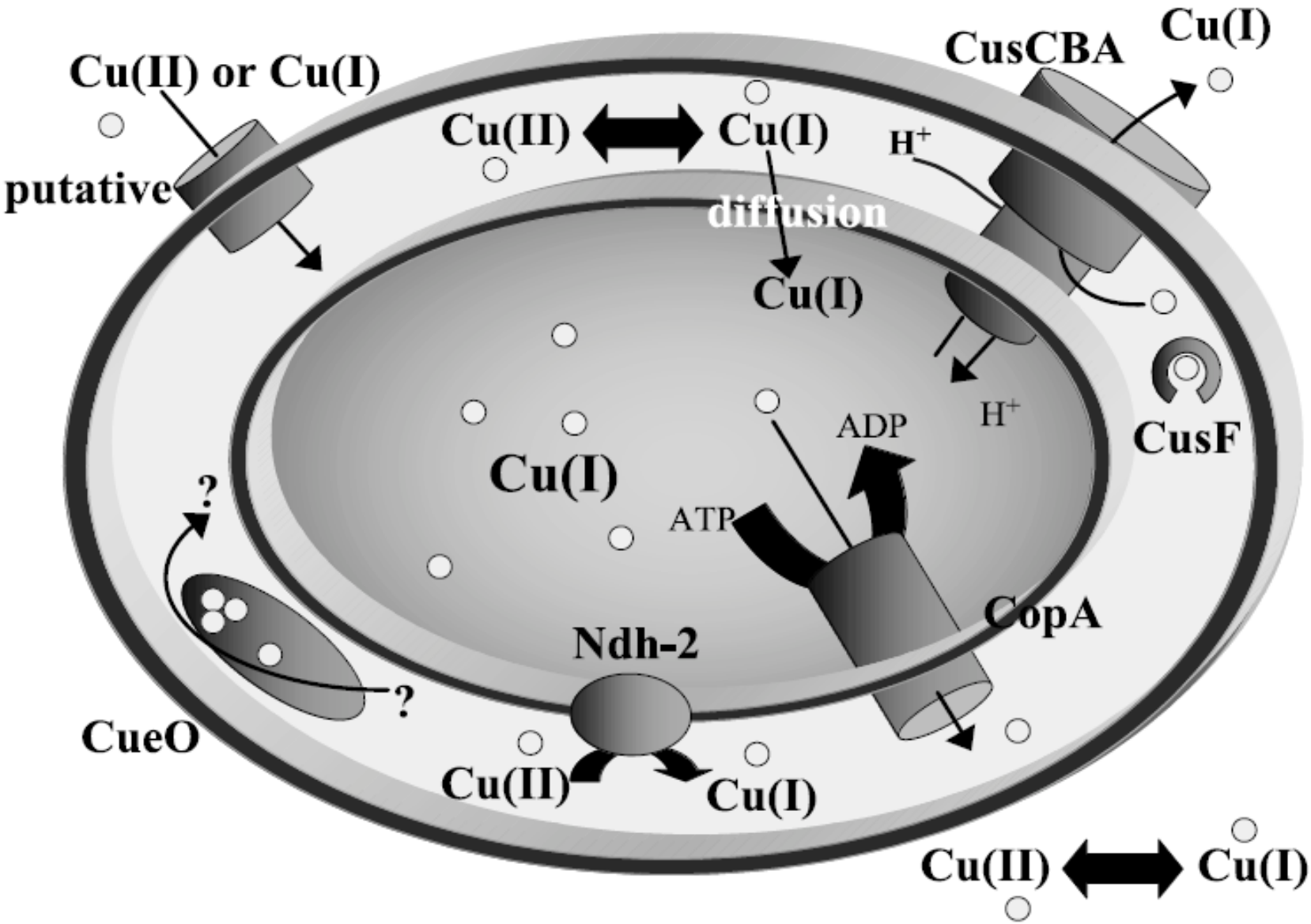
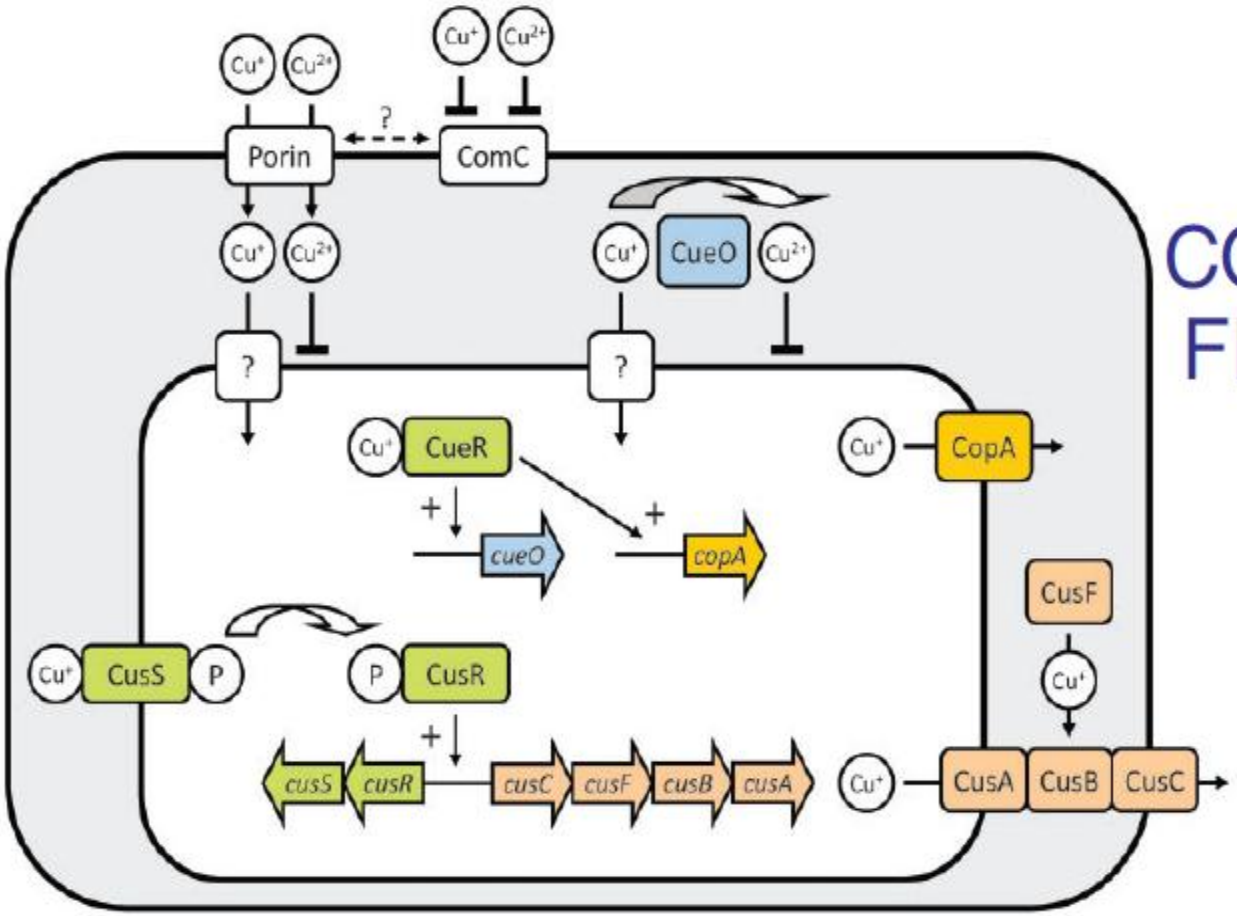

Cu(II) or Cu(I)
putative
Cu(II)
Cu(I)
CusCBA
Cu(I)
H+
diffusion
Cu(I)
H+
CusF
ADP
Cu(I)
ATP
?
CopA
Ndh-2
?
CueO
Cu(II)
Cu(I)
Cu(II)
Cu(I)

Table 1
Essential elements of copper homeostasis in *E. coli*

Homeostatic mechanism	Regulated by	Function
CopA	CueR (sensing cytoplasmic Cu[I]) and CpxR (sensing cell envelope stress)	Detoxification of cytoplasmic Cu[I]
CusCFBA	CusRS (two-component regulation system, sensing periplasmic Cu[I])	Detoxification of periplasmic (and possibly cytoplasmic) Cu[I]
CueO	CueR (sensing cytoplasmic Cu[I])	Protection of periplasmic proteins from copper-induced toxicity
PcoABCD	PcoRS, (two-component regulation system, sensing periplasmic Cu[I]) (and CusRS)	Protection from extreme periplasmic copper stress
PcoE	CusRS (PcoRS)	Periplasmic copper chaperone, copper binding



COLOUR FIGURE

Fig. 1. Copper homeostasis in *E. coli*. Cu^{+} and Cu^{2+} ions enter the periplasm (shown in grey), probably via porins spanning the outer membrane. ComC lowers the permeability of the outer membrane for copper ions. It is unknown whether ComC interacts with porins. At low ambient copper concentrations, *comC* transcription is repressed by ComR (not shown). Cu^{+} ions proceed from the periplasm into the cytoplasm by an unknown mechanism, while Cu^{2+} ions do not cross the cytoplasmic membrane. Copper efflux involves the ATPase CopA and the multicomponent system CusCFBA. The multicopper oxidase CueO oxidizes periplasmic Cu^{+} to Cu^{2+}. For further details, see text.

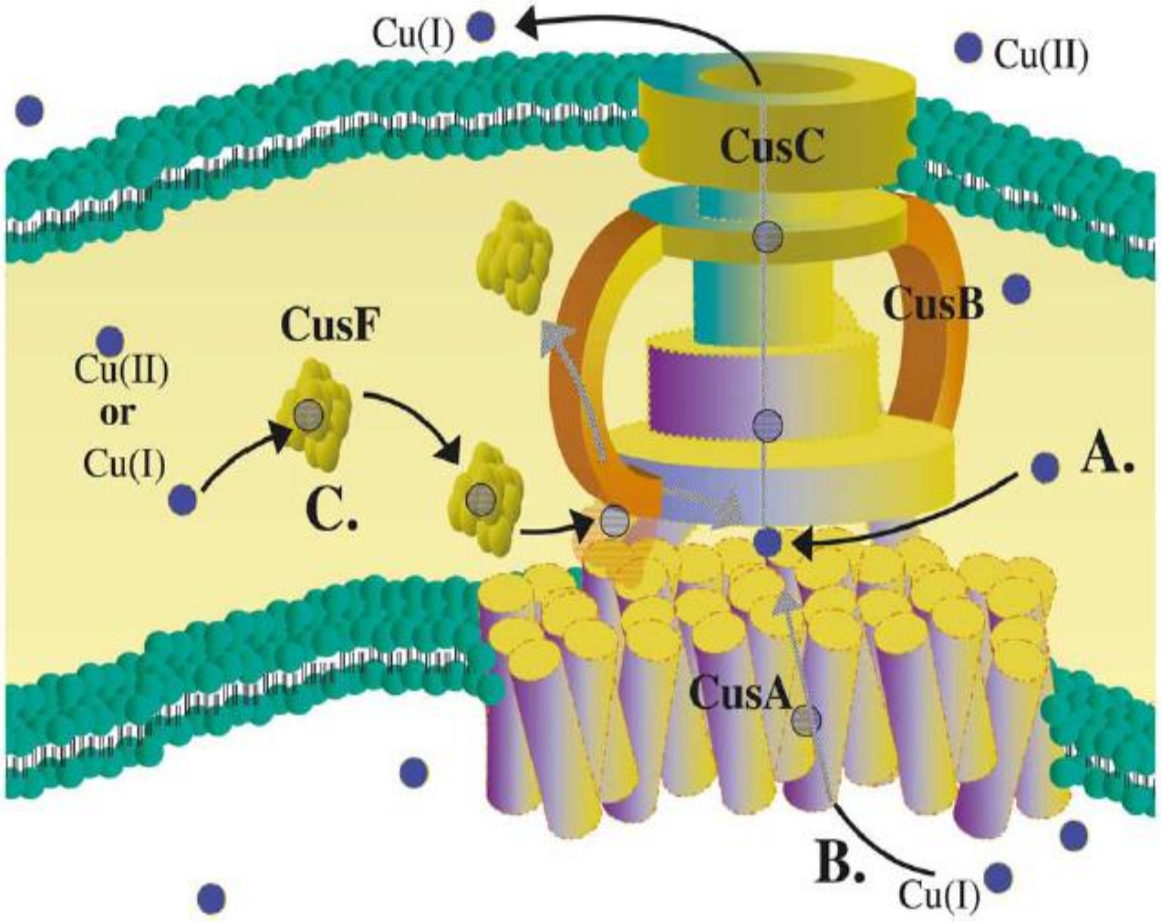


Fig. 2. Functional model of the Cus efflux complex. The four-part Cus complex consists of the inner membrane pump CusA, the periplasmic protein CusB and the outer membrane protein CusC forming a channel bridging the periplasmic space. Entry of copper may occur from the periplasm (A), from the cytoplasm (B) or via the copper-binding chaperone CusF from the periplasm (C).

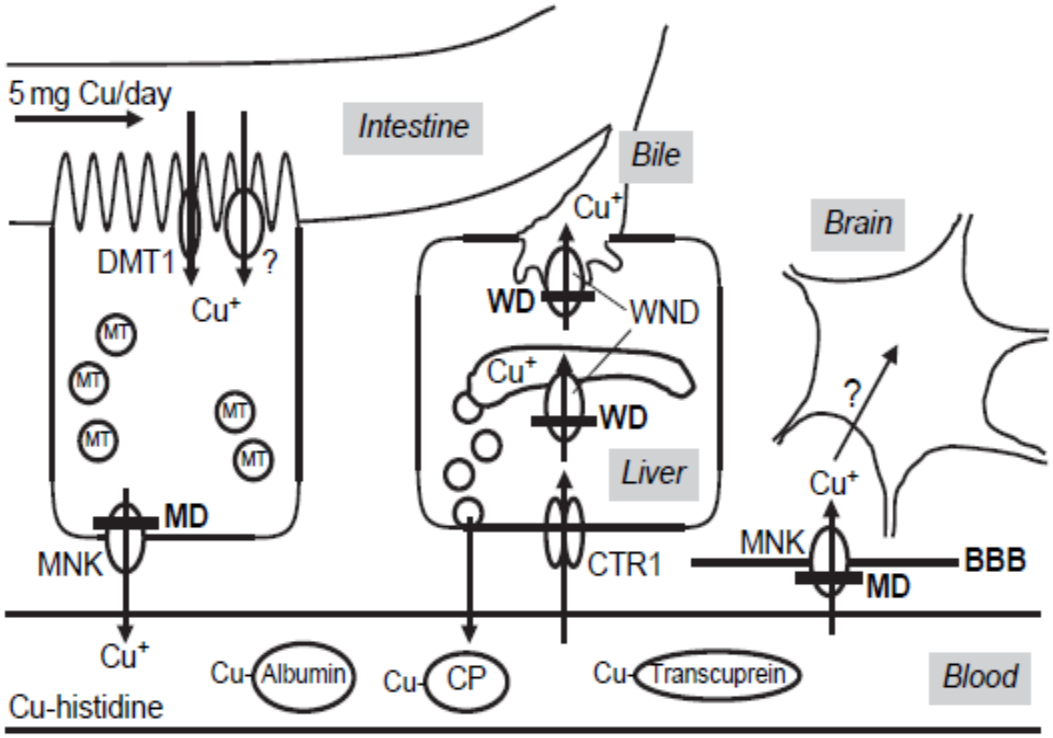


FIGURE 1. Schematic drawing of the key elements of copper homeostasis and defects in Wilson and Menkes diseases. Dietary copper in the intestine may be taken up by intestinal cells via DMT1 and also by an as yet unidentified system. Metallothioneins (MT) can buffer excess copper inside these and other cells. The Menkes copper ATPase (MNK) exports copper from intestinal cells into the circulation. In the blood, copper can form complexes with small molecules like histidine and is bound to proteins such as albumin or transcuprein. The liver takes up circulating copper by a first-pass process, probably involving CTR1. In hepatocytes (liver cells), copper is transported into the trans-Golgi network by the Wilson ATPase (WND) for incorporation into ceruloplasmin (CP). Ceruloplasmin, a multicopper ferroxidase with a role in iron homeostasis, is secreted into the circulation via the secretory pathway. Excess copper is excreted into the bile by hepatocytes. This process also requires the activity of the Wilson ATPase, which undergoes copper-induced trafficking from a trans-Golgi to a periplasmic location. The transport of copper across the blood–brain barrier (BBB) appears to be catalyzed by the Menkes ATPase expressed in cerebrovascular endothelial cells. The lack of function of the Menkes copper ATPase in Menkes disease (—MD) leads to copper accumulation in the intestine and concomitant copper deficiency in most tissues. Systemically administered copper cannot be transported to the brain. A defect in the Wilson copper ATPase in Wilson disease (—WD) results in copper accumulation in hepatocytes.

TABLE 1. A Postulated Spectrum of Copper Metabolism (from Aggett, 1999)

Dose range	Approximate daily intakes	Health outcomes
		Death
		Gross dysfunction and disturbance of metabolism of other nutrients; hepatic "detoxification" and homeostasis overwhelmed
Toxic	>5.0 mg/kg body weight	Gastrointestinal metallothionein induced (possible differing effects of acute and chronic exposure);
	100 µg/kg body weight	Plateau of absorption maintained; homeostatic mechanisms regulate absorption of copper
Adequate	34 µg/kg body weight	Hepatic uptake, sequestration and excretion effect homeostasis; glutathione-dependent uptake of copper; binding to metallothionein; and lysosomal excretion of copper
	11 µg/kg body weight	Biliary excretion and gastrointestinal uptake normal
	9 µg/kg body weight	Hepatic deposit(s) reduced; conservation of endogenous copper; gastrointestinal absorption increased
Deficient	8.5 µg/kg body weight	Negative copper balance
	5.2 µg/kg body weight	Functional defects, such as lysyl oxidase and superoxide dismutase activities reduced; impaired substrate metabolism;
	2 µg/kg body weight	Peripheral pools disrupted; gross dysfunction and disturbance of metabolism of other nutrients; death

METHODS TO DETECT PESTICIDES

Chromatographic Methods → Gas Chromatography (GC) & High Performance Liquid Chromatography (HPLC) , coupled with mass spectrometry (MS)

- Sensitive and realiable
- Complex
- A lot f time to realize the analysis
- Required highly trained technicians
- not in-field / on-site detection

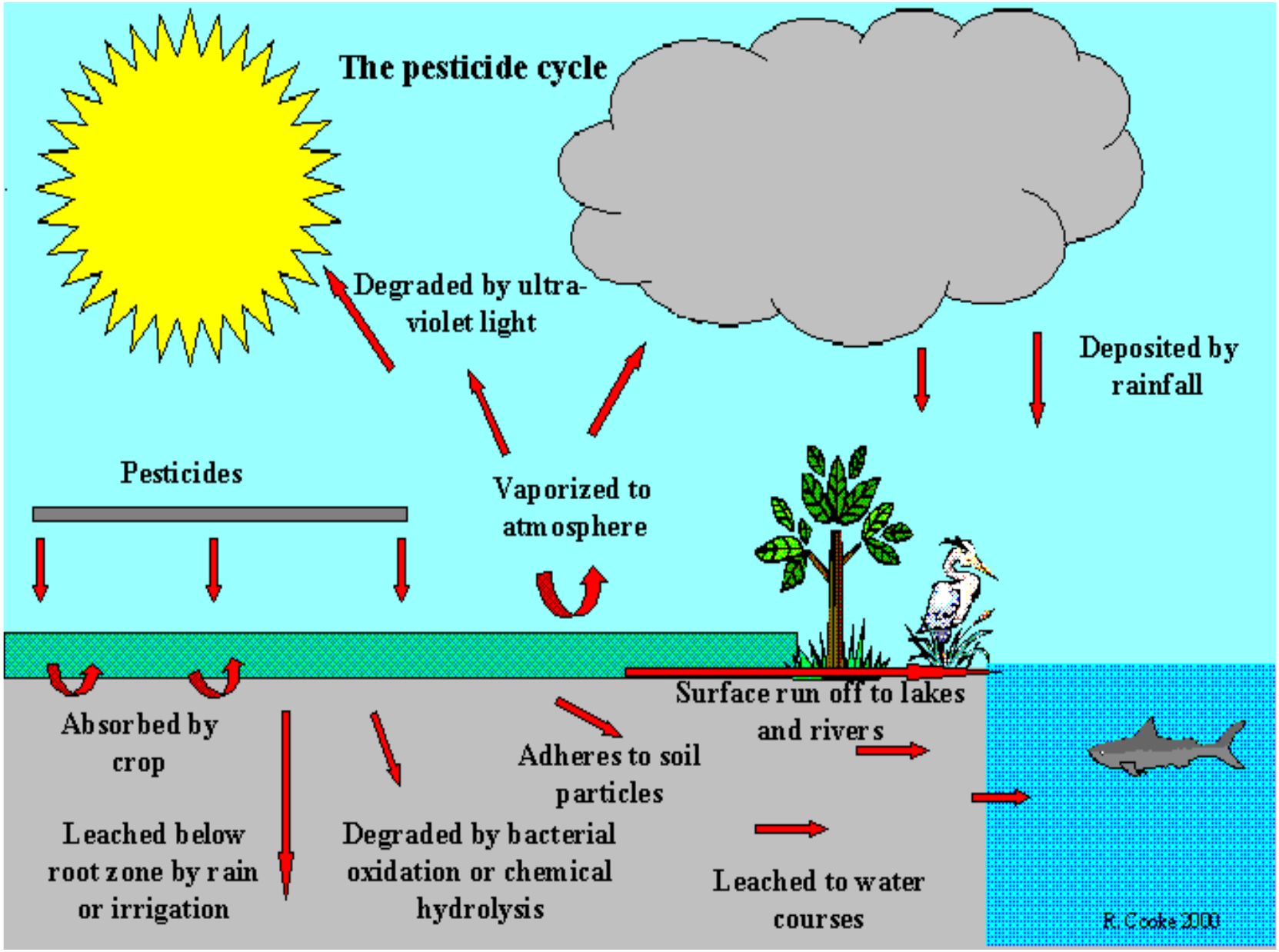
METHODS TO DETECT COPPER IN WATER

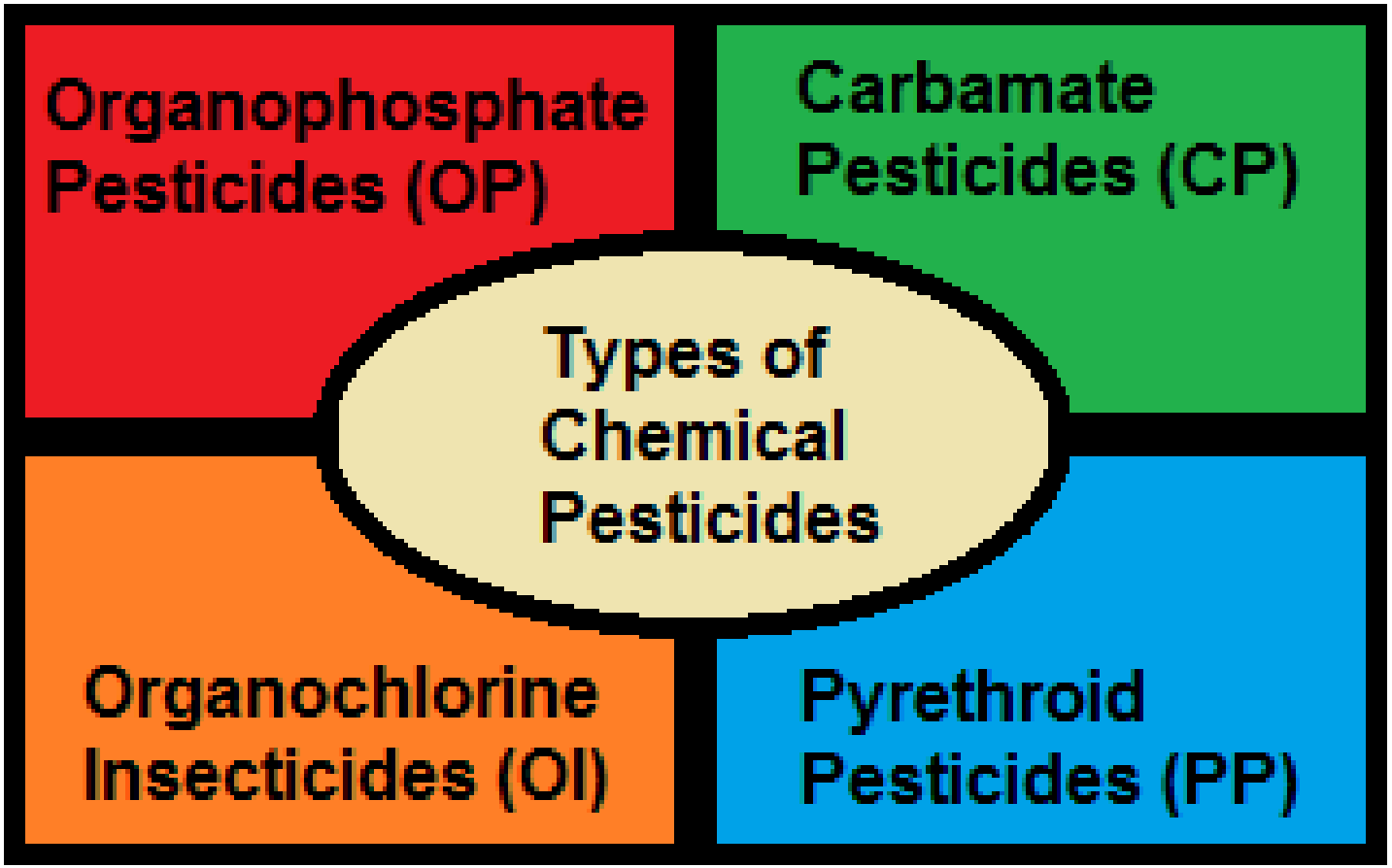
The *Copper reagents and applications* table lists proprietary reagents and applications.

Table 1 Copper reagents and applications

Reagent	Form measured		Application
	Without pretreatment	With digestion	
CuVer 1™[1]	Free	Total recoverable	water, wastewater
CuVer 2™	Total dissolved copper	Total recoverable	
Free copper reagent	Free	Total recoverable	hard water, wastewater, seawater
Porphyrin reagents	Free	Total recoverable	extremely low levels in water, wastewater and seawater

[1] CuVer is a trademark of Hach Company.


The pesticide cycle
Degraded by ultra-violet light
Deposited by rainfall
Pesticides
Vaporized to atmosphere
Surface run off to lakes and rivers
Absorbed by crop
Adheres to soil particles
Leached below root zone by rain or irrigation
Degraded by bacterial oxidation or chemical hydrolysis
Leached to water courses
R. Cooke 2000


Organophosphate Pesticides (OP)
Carbamate Pesticides (CP)
Types of Chemical Pesticides
Organochlorine Insecticides (OI)
Pyrethroid Pesticides (PP)

COPPER FOR CROP PRODUCTION

- **Classified as a micronutrient**

Role in plant growth :

- promotes seed production and formation
- plays an essential role in chlorophyll formation
- essential for proper enzyme activity

COPPER IN SOILS

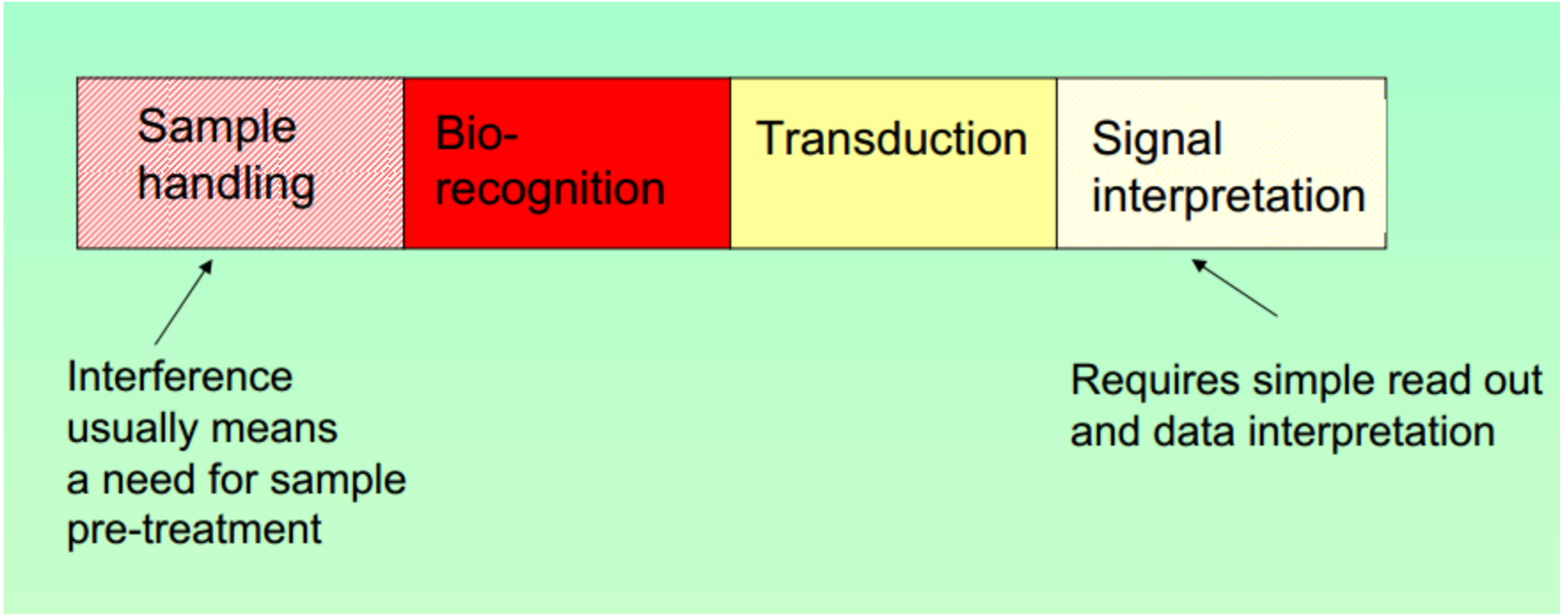
- Copper is not mobile in soils

Available Cu can vary from 1 to 200 ppm in both mineral and organic soils

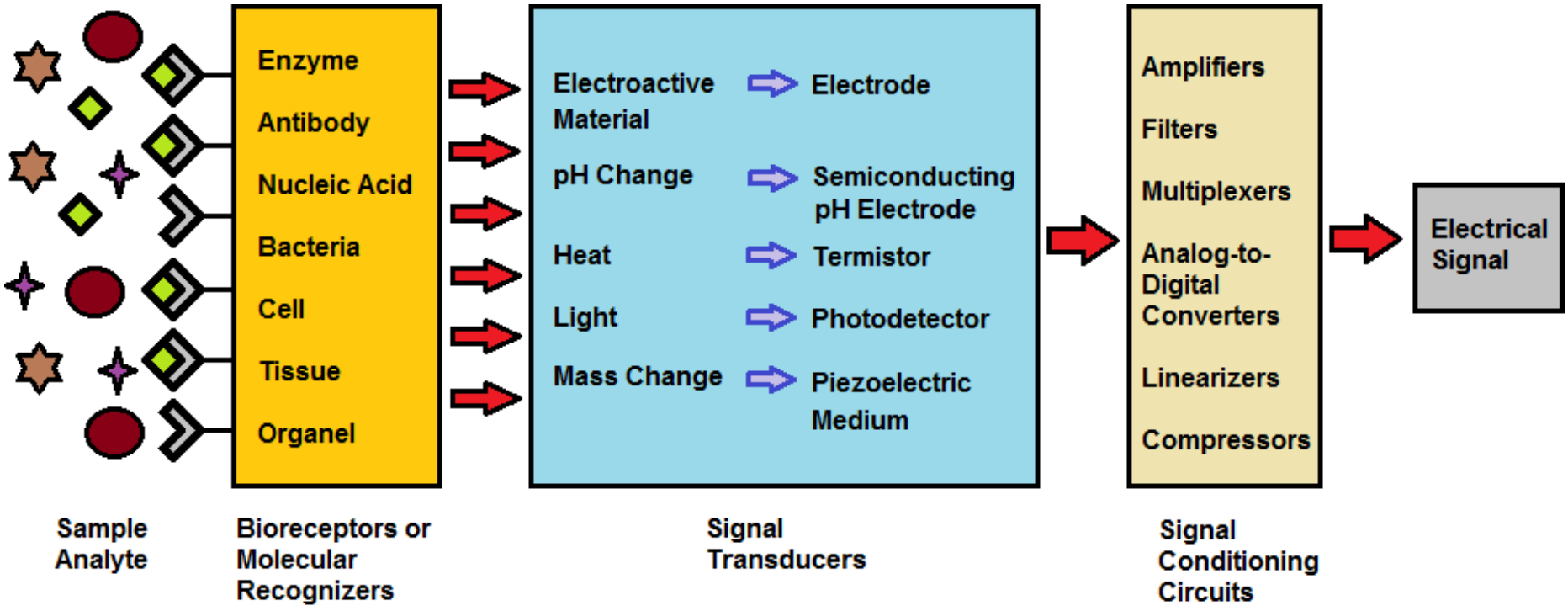
- Related to soil pH  availability Cu 

Crop	Plant Part Sampled	Time of sampling	Deficient	Low	Sufficient	High	Excessive
					----------ppm Cu----------		
corn	ear leaf	silking	<2.0	2.0-5.0	5.1-20.0	20.1-50	>50
soybeans	top trifoliate	flowering	<5.0	5.0-9.0	9.1-30.0	30.1-50	>50
wheat	top leaves	boot	<3.0	3.0-5.0	5.1-20.0	20.1-50.0	>50

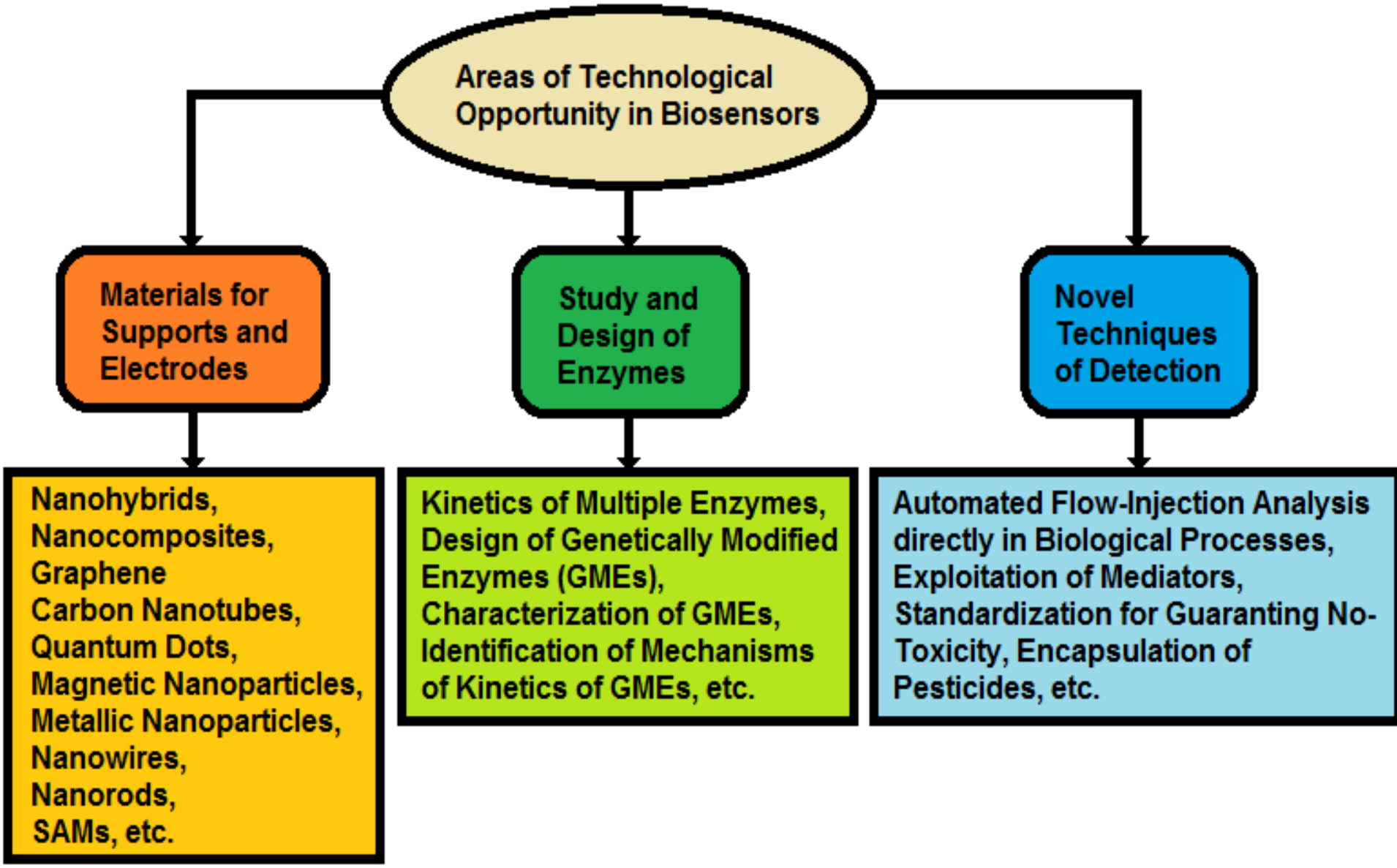
FUTURE DEVELOPMENT



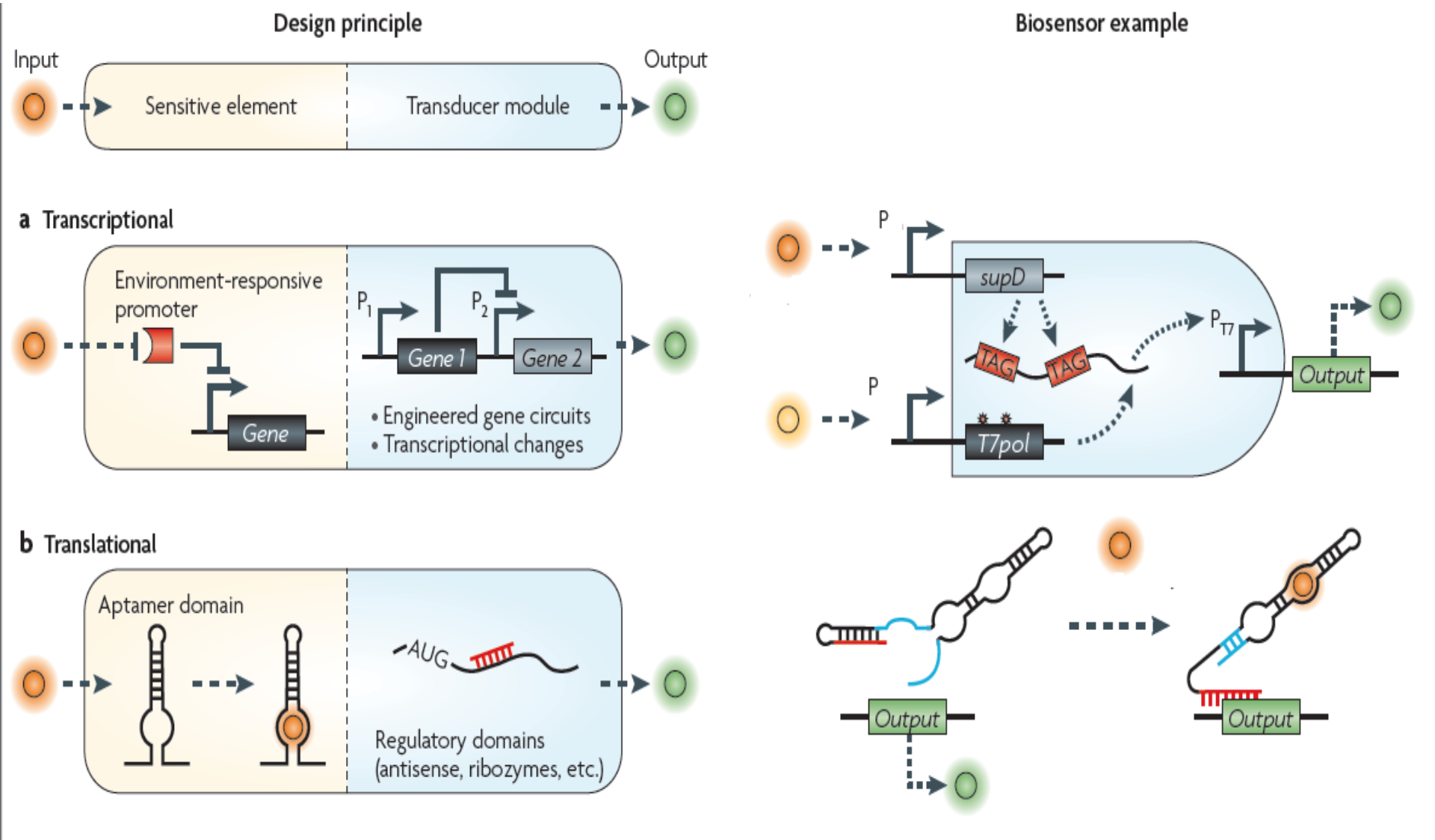
FUTURE DEVELOPMENT



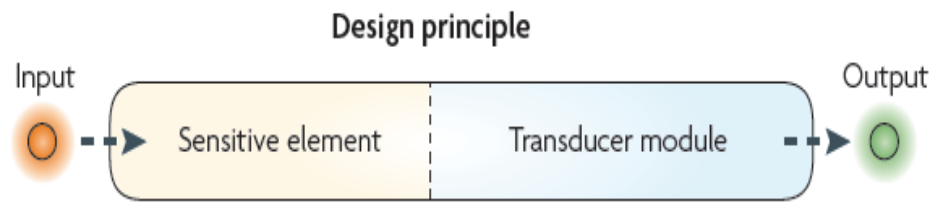
FUTURE DEVELOPMENT

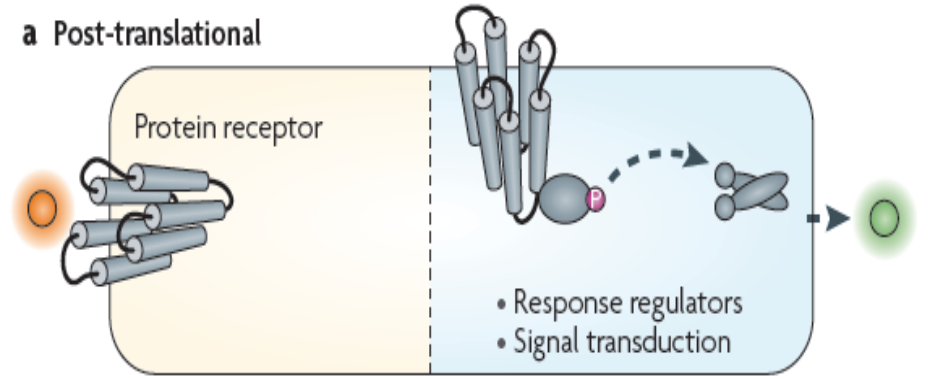


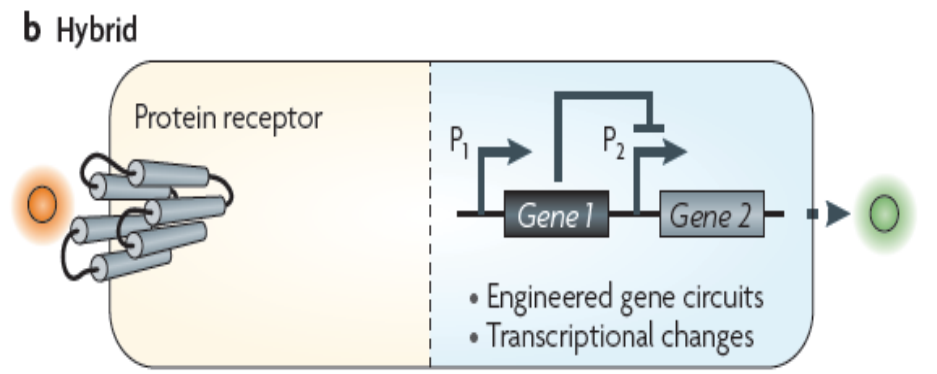
CUPID BIOSENSOR

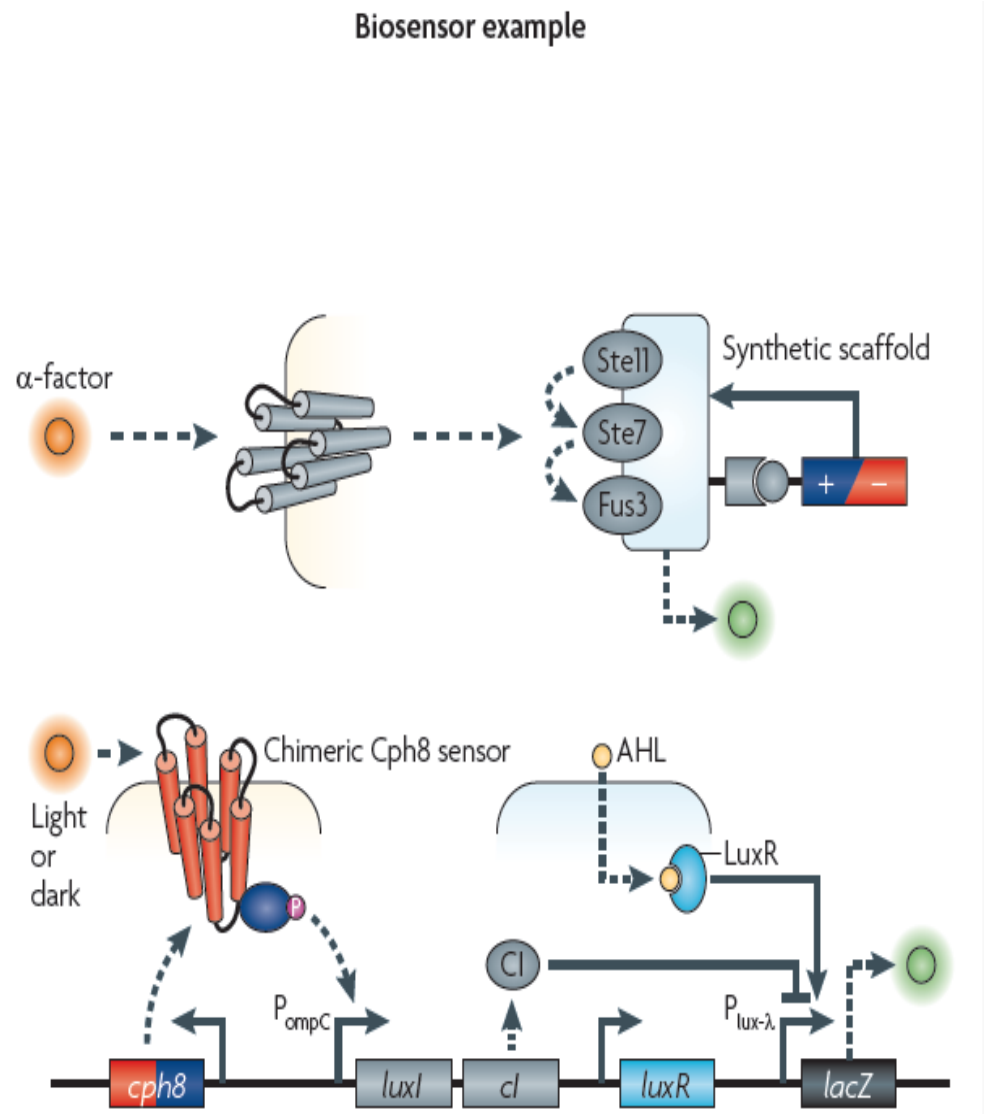


CUPID BIOSENSOR









Desain biologi sintetik CUPID *E. coli* ini menggunakan Standar RFC 10, yaitu desain standar perangkaian *BioBrick Parts* dengan menggunakan enzim restriksi dan ligasi yang membentuk situs *scar* atau M

Prefix: GAATTCGCGGCCGCTTCTAGAG
CTTCCGCGCCGGCGAAGATCTC

Suffix: TACTAGTAGCGGCCGCTGCAG
ATGATCATCGCCGGCGACGTC

EcoRI
XbaI
SpeI
PstI
NotI

Prefix cut with **XbaI**:
...GCTT^CTAG AG
...CGAA GATC^TC

Suffix cut with **SpeI**:
TA^CTAG TAGC...
AT GATC^ATCG...

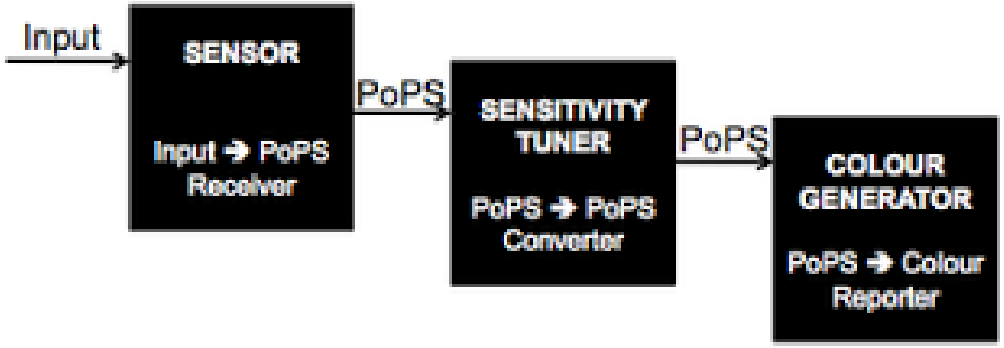
SpeI + XbaI
...TA^CTAG AG...
...AT GATC^TC...

http://partsregistry.org/Help:BioBrick_Prefix_and_Suffix

PENGEMBANGAN RANCANGAN

- Bakteri *E.coli* dibuat dalam bentuk *blotter*, sehingga mudah untuk diaplikasikan di lapangan
- Sistem CUPID dikembangkan menjadi metode deteksi kuantitatif

  Level induksi (konsentrasi Cu) → level ekspresi
- Sistem CUPID dikembangkan untuk menjadi detektor senyawa logam lainnya, misalnya merkuri


Input
SENSOR
Input → PoPS Receiver
PoPS
SENSITIVITY TUNER
PoPS → PoPS Converter
PoPS
COLOUR GENERATOR
PoPS → Colour Reporter